Bull. Environ. Contam. Toxicol. (1995) 55:469–472

Environmental Contamination and Toxicology

Comparison of Three Lipid Extraction Methods for Fish

M. E. Honeycutt,[1] V. A. McFarland,[1] D. D. McCant[2]

[1]U.S. Army Engineer Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, Mississippi 39180, USA
[2]AScI Corporation, 1720 Clay Street, Vicksburg, Mississippi 39180, USA

Received: 11 September 1994/Accepted: 15 January 1995

The role of lipids as the major compartment for neutral organic chemical partitioning in organism tissues has been well documented (Esser 1986; Roberts et al. 1977; Connell 1988; Schneider 1982). Lipid normalization is used in the calculation of accumulation factors (AF), which express the equilibrium distribution of neutral chemicals between sediments and aquatic biota (Ankley et al. 1992; Ferraro et al. 1990, 1991; Lake et al. 1990; McFarland et al. 1994; Young et al. 1991). A screening test used to estimate the bioaccumulation potential of neutral chemicals associated with dredged sediments relies on equilibrium partitioning to organism lipids (USEPA/USACE 1991). Additionally, the USEPA seeks to promulgate sediment quality criteria (SQC) that will require lipid normalization of data (USEPA 1993). Thus, lipid content of aquatic organism tissues is becoming an increasingly important parameter in environmental regulation involving contaminants in sediments.

No standardized method exists for lipid determinations in environmental tissue samples. Typically, analysts either reserve an aliquot of a residue-analysis tissue extract for lipid analysis, or analyze a separate tissue sample for lipids concurrently. In the former case, hexane:acetone or dichloromethane are commonly used as solvents (Ryan et al. 1985; Schwartz et al. 1993). In the latter case, the chloroform:methanol (Bligh-Dyer) method is commonly used, as it is specifically intended for lipid analysis and is routinely used to measure the lipid content of foods (Bligh and Dyer 1959). Often, the amount of tissue used in either of the above cases may vary due to differing amounts of tissue required (or available) for chemical analysis, or to the amount of sample remaining for lipid analysis after that required for chemical analysis has been taken. Knowledge of the variability that may be introduced due to sample size or solvent used is required in order to compare lipid-normalized data obtained from different studies.

The purpose of this study was to assess these sources of variability by comparing percent lipid determinations made on different sample sizes of the same homogenized fish tissue. Three lipid extraction methods (Bligh-Dyer, hexane:acetone and dichloromethane) and six sample sizes representing a two hundred-fold range of tissue weights were compared. While other extraction methods, e.g., petroleum ether, pentane, acetonitrile, etc., are used to some extent, in our experience the three methods in the present study are the most often used, especially in the regulatory realm.

Correspondence to: M. E. Honeycutt

MATERIALS AND METHODS

Nine kilograms of frozen commercial whiting fish was purchased from a local supermarket, skinned and filleted. The fillets were homogenized with a Waring blender, divided into 100-g fresh weight aliquots and stored at -80° C until use. For each lipid extraction method, four replicate tissue samples were used for each tissue sample size. All procedures were performed at room temperature.

Tissue samples of 0.5, 1, 5, 10, 50, or 100 g were analyzed for lipid content using the Bligh-Dyer method (Bligh and Dyer 1959). The samples were homogenized for 2 min using either a Waring blender or a Polytron homogenizer (depending on sample size) with chloroform and methanol in the proportion of 1 g tissue:1 mL chloroform:2 mL methanol. Solvent volumes were adjusted for each sample size to maintain the same proportions. An additional equivalent amount of chloroform was added and the mixture which was homogenized for 30 sec. Deionized water (1 mL water/1 g tissue) was then added to the mixture, and it was homogenized again for 30 sec. The final mixture proportion was 1 g tissue:2 mL chloroform:2 mL methanol:1 mL deionized water. The mixture was filtered through Whatman No. 1 filter paper, and the remaining tissue was homogenized for 2 min with another 1 mL chloroform/1 g tissue. After filtering the mixture again, the combined filtrate was transferred to a graduated cylinder and allowed to separate. Lipid content was determined gravimetrically by measuring triplicate aliquots of the chloroform layer into tared containers, allowing the solvent to evaporate, and weighing. Percent lipid determinations were then calculated.

For dichloromethane extraction, tissue samples of 0.5, 1, 5, or 10 g were placed into 25 or 150 mL screw-capped centrifuge tubes according to sample size along with one to two times the tissue weight of anhydrous sodium sulfate. Dichloromethane in a proportion of 5 mL to 1 g tissue was added to the samples, which were rotated end over end for 18 to 24 hr. The mixture was filtered through Whatman No. 1 filter paper and percent lipid determinations were made for triplicate aliquots of the dichloromethane extracts as with the Bligh-Dyer method.

For hexane:acetone extraction, tissue samples of 0.5, 1, 5, 10, or 50 g were analyzed for lipid content by homogenizing each sample three times with 20 mL hexane:acetone (1:1, v/v) for 2 min using a Polytron homogenizer. The three extracts were filtered and pooled. Percent lipids were calculated on triplicate extract aliquots as with the Bligh-Dyer method.

All data were analyzed using PC SAS® (SAS Institute 1988). Two-way analysis of variance was performed using PROC GLM and mean comparisons were made using Fisher's Protected LSD. The normality assumption was tested using the Shapiro-Wilk's test and homogeneity of variances was assessed using Levene's test (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Percent lipid data for the three methods used are listed in Table 1. The hexane:acetone method was impractical for use with the 100 g sample size. Similarly, the dichloromethane method could not be performed using sample sizes of 50 and 100 g.

The data indicate that sample size has a significant effect on lipid analysis results regardless of method. The whiting tissue apparently had a percent lipid value of a little more than one percent (median = 1.12%, IQR = 0.923 - 1.37%). The data were non-normally distributed and all three methods yielded results encompassing this value at

one or more sample weights. Lipid determinations made using the 0.5 and 1 g sample weights were the most variable and yielded significant differences among the methods, while the 5 and 10 g sample sizes were not significantly different. The Bligh-Dyer method generally gave higher percent lipid values, yielding significantly higher results for the 1 g sample size.

Table 1. Mean percent lipids ± SE on a fresh-weight basis of commercial whiting fish fillets.

Sample Size (g)	Method		
	Bligh-Dyer	Hexane:Acetone	Dichloromethane
0.5	2.07 ± 0.20 A[a] b[b]	1.29 ± 0.12 A b	5.77 ± 0.37 A a
1	1.47 ± 0.29 B a	0.63 ± 0.05 C b	0.28 ± 0.05 B b
5	1.12 ± 0.26 B a	0.93 ± 0.14 B a	0.71 ± 0.11 BC a
10	1.06 ± 0.02 B a	0.92 ± 0.06 BC a	1.14 ± 0.37 C a
50	1.25 ± 0.12 B a	1.00 ± 0.08 AB a	–
100	1.39 ± 0.19 B	–	–

[a]For a given method, sample size means followed by the same upper case letter are not significantly different from each other ($p < 0.05$).
[b]For a given sample size, method means followed by the same lower case letter are not significantly different from each other ($p < 0.05$).

Randall et al. (1991) found a 3.5-fold variation among several extraction methods which included acetonitrile extraction with pentane partitioning, acetone extraction with hexane partitioning, Bligh-Dyer, and acetonitrile extraction using sample sizes of 1 to 5 g. Results from this study suggest that larger sample sizes (5 to 10 g) may yield less variable results and would be comparable using the three methods investigated.

The 0.5 g sample size for all three extraction methods yielded an aberrantly high percent lipid, indicating that this sample size is below the limit of practical application of the three methods. Samples < 5 g should probably be analyzed using a micromethod such as the method described by Gardner et al. (1985).

Sample sizes of 5 to 10 g were optimal for the three lipid extraction methods studied, and produced similar results for all three methods. The 100 g sample size called for in the original Bligh-Dyer method is usually impractical for environmental studies involving small organisms, and is not necessary. However, if less than 5 g of tissue is available, a micromethod should be used.

Acknowledgments. This research was supported by the Long-Term Effects of Dredging Operations Program of the U.S. Army Corps of Engineers. Permission was granted by the Chief of Engineers to publish this information.

REFERENCES

Ankley GT, Cook PM, Carlson AR, Call DJ, Swenson JA, Corcoran HF, Hoke RAH (1992) Bioaccumulation of PCBs from sediments by oligochaetes and fishes: Comparison of laboratory and field studies. Can J Fish Aquat Sci 49:2080-2085

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911-917

Connell DW (1988) Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. Rev Environ Contam Toxicol 101:121-154

Esser HO (1986) A review of the correlation between physicochemical properties and bioaccumulation. Pestic Sci 17:265-276

Ferraro SP, Lee II H, Smith LM, Ozretich RJ, Specht DT (1991) Accumulation factors for eleven polychlorinated biphenyl congeners. Bull Environ Contam Toxicol 46:276-283

Ferraro SP, Lee II H, Ozretich RJ, Specht DT (1990) Predicting bioaccumulation potential: A test of a fugacity-based model. Arch Environ Contam Toxicol 19:386-394

Gardner WS, Frez WA, Chichocki EA, Parrish CC (1985) Micromethod for lipid analysis in aquatic invertebrates. Limnol Oceanogr 30:1099-1105

Lake JL, Rubinstein NI, Lee II H, Lake CA, Heltsche J, Pavignano S (1990) Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ Toxicol Chem 9:1095-1106

McFarland VA, Feldhaus J, Ace LN, Brannon JM (1994) Measuring the sediment/organism accumulation factor of PCB-52 using a kinetic model. Bull Environ Contam Toxicol 52:699-705

Randall RC, Lee II H, Ozretich RJ, Lake JL, Pruell RJ (1991) Evaluation of selected lipid methods for normalizing pollutant bioaccumulation. Environ Tox Chem 10:1431-1436

Roberts JR, deFrietas ASW, Gidney MAJ (1977) Influence of lipid pool size on bioaccumulation of the insecticide chlordane by northern redhorse suckers (*Moxostoma macrolepidotum*). J Fish Res Board Can 34:89-97

Ryan JJ, Schecter A, Lizotte R, Sun W, Miller L (1985) Tissue distribution of dioxins and furans in humans from the general population. Chemosphere 14:929-932

SAS Institute Inc (1988) SAS/STAT™ procedures guide, release 6.03 ed. Cary, North Carolina

Schneider R (1982) Polychlorinated biphenyls (PCBs) in cod tissues from the Western Baltic: significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. Meeresforsch 29:69-79

Schwartz TR, Tillitt DE, Feltz KP, Peterman PH (1993) Determination of mono- and non-o,o'-chlorine substituted polychlorinated biphenyls in aroclors and environmental samples. Chemosphere 26:1443-1460

Snedecor GW, Cochran WG (1989) Statistical methods, 8th ed. Iowa State University Press, Ames, Iowa

United States Environmental Protection Agency (1993) Technical basis for deriving sediment quality criteria for nonionic organic contaminants for the protection of benthic organisms by using equilibrium paritioning. EPA-822-R-93-011, Office or Water (WH-553), Washington, DC 20460

United States Environmental Protection Agency/United States Army Corps of Engineers (1991) Evaluation of dredged material proposed for ocean disposal: testing manual. EPA-503/8-91/001, Department of the Army, U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS 39180

Young DA, Mearns AJ, Gossett RW (1991) Bioaccumulation of *p,p'*-DDE and PCB 1254 by a flatfish bioindicator from highly contaminated marine sediments of Southern California. In: Baker RA (ed) Organic Substances and Sediments in Water: Biological Processes, American Chemical Society, Washington, DC, pp 159-169